# Biodiversity and Spatial Concordance of an *in situ* System for Uranium Bioreduction

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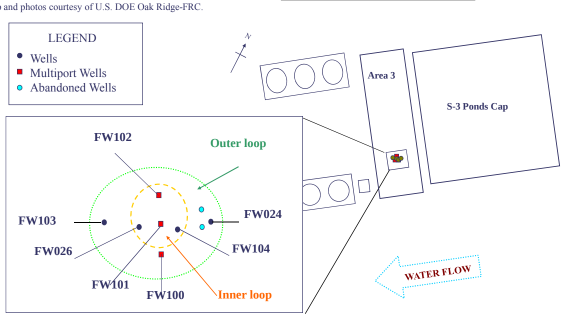
## ABSTRACT

The elucidation of how populations of interest interact in a given community and how the community responds to stress and perturbations can help infer the interplay between stress pathways and gene networks that help optimize bacterial biochemistry. A goal of VIMSS is to characterize the responses of bacterial communities at multiple levels of resolution in order to understand biochemical capacity at DOE waste sites. The current work uses a series of re-circulating wells that create a subsurface bioreactor to stimulate microbial growth for *in situ* U(VI) immobilization (Wu et al. ES&T 41:5716-5723). Bacterial community dynamics were investigated in a series of re-circulating wells that created a subsurface "bio-reduction zone" to stimulate growth with ethanol for *in situ* bioremediation of U(VI) at the Field Research Center of the U.S. Department of Energy, Oak Ridge, TN. Different experiments were conducted to alter the subsurface environment to better understand strategies that would improve the remediation process. Within this framework, the interrelationships between the biogeochemistry were studied in order to characterize the community and ecosystem ecology with respect to microbiology of an engineered system. Bacterial community composition and structure of groundwater samples were analyzed via clone libraries of partial SSU rRNA genes. UniFrac analyses showed that the bacterial community in each of the wells developed changes during the bioremediation process, and the changes could be attributed to the variations along temporal and spatial scales. Relationships between community diversity and ecosystem function were idiosyncratic, and these results suggested the population distributions depended on the particular conditions under which the local landscape was investigated. Principal component analysis showed that nitrate, uranium, sulfide, sulfate, and COD were strongly associated with particular bacterial populations. Sequences closely related to nitrate-reducing bacteria were predominant during the initial phase of the remediation process, but sequences representative of sulfate-reducers (*Desulfovibrio* and *Desulfosporosinus* spp.) and metal-reducers (*Geobacter* spp.) were detected at higher levels as uranium levels declined. Ultimately, sequences associated with sulfate-reducing populations predominated. Uranium levels declined below EPA drinking water standards, and community composition and structure were similar in both treatment wells after approximately 1.5 y despite going through different transitions. In addition, when engineering controls were compared to the community structure and composition via canonical ordinations, population distributions could be related with dissolved oxygen control and the presence of bio-stimulant. During the bio-stimulation, population distributions followed geochemical parameters, and these results indicated that bacteria exhibited distributions at the landscape scale in concordance with predictable geochemical factors. The data indicated that relationships between community structure and ecosystem function were idiosyncratic, but temporal and spatial concordance were eventually observed for the two bio-stimulated wells. The strong associations between particular environmental variables and certain population distributions will provide insights into establishing practical and successful remediation strategies in radionuclide-contaminated environments with respect to engineering controls and ecosystem function.

**Map of the Oak Ridge Field Research Center.**

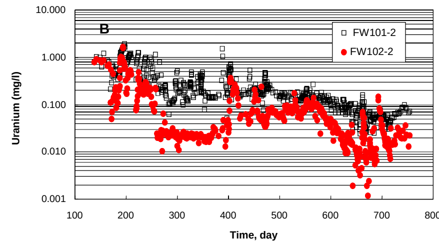

**View of S-3 ponds before and after capping in to a parking lot.**

Map and photos courtesy of U.S. DOE Oak Ridge-FRC.

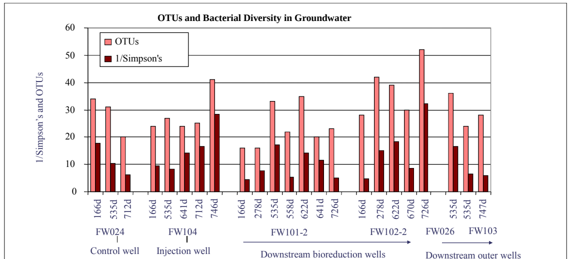



**Area view of well design at Area 3.** A series of re-circulating wells establish a subsurface bioreactor to stimulate microbial growth for *in situ* U(VI) immobilization. Well FW-104 is the injection well for the electron donor (ethanol); well FW-026 is the extraction well for the recirculation loop; well FW-101 is the center of biostimulation; and FW-024 and FW-103 are upstream and downstream wells, respectively.

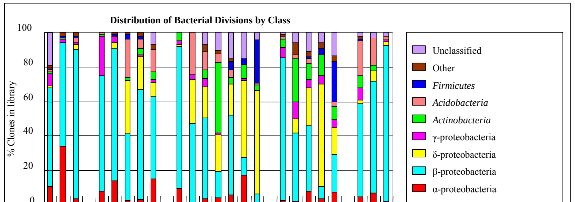
## RESULTS



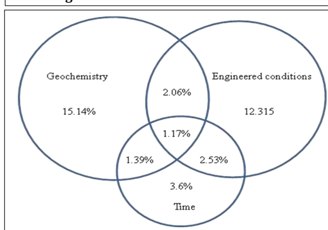

Uranium U(VI) concentrations in groundwater of monitoring wells over time in the bio-reduction zone . Nitrate levels were below 0.20 mM between 200 and 350 d, peaked to 1.5 mM between 350 and 500 days, and fell below 0.1 mM after day 500.




**Diversity and OTUs of each well.** The diversity index, 1/Simpson's, and OTUs were calculated by generating a distance matrix in MEGA version 3.1 and importing into DOTUR. The diversity in the injection well, FW104, continued to increase, while fluctuations in diversity occurred in the two bioreduction wells, FW101-2 and FW102-2, during the bioremediation process. The two outer wells, FW103 and FW026, which were not stimulated for bacterial growth did not experience much change in diversity. A decline in bacterial diversity was observed in the control well, FW024. A similar pattern was observed in OTU distribution.




**Distribution (% of the total) of clones from the groundwater in each well over time according to all observed bacterial divisions.**




Percent of total variance in the bacterial abundance data due to environmental factors explained by geochemistry, engineered conditions, and time variables, as well as variance explained by each combination of factors. Figure is not drawn to scale.

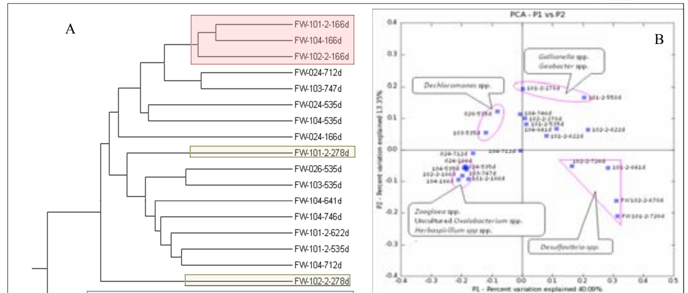
## CONCLUSIONS

•The data suggested that the population distributions depended on the particular conditions under which the local landscape was investigated

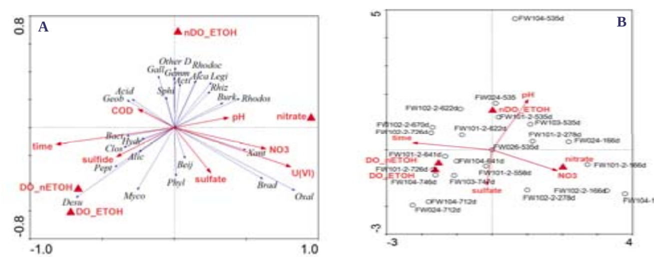
•Relationships between community structure and ecosystem function were idiosyncratic – yet temporal and spatial concordance were eventually observed for the two bio-stimulated wells

•Geochemical and engineering controls could not explain a portion of the biological variability

## RESULTS




UniFrac statistical comparison of groundwater bacterial communities based upon phylogenetic comparisons. (A) UPGMA tree based upon UniFrac distances showing the overall phylogenetic relationships of each sampled community at a given sampling time. (B) Principal coordinate analysis (PCoA) of UniFrac distances resulted in a clustering pattern similar to that observed by UPGMA. The circled groupings indicate the bacterial communities that were grouped.




**A.** Redundancy analysis (RDA) ordination diagram (biplot) summarizing the effects of enironmental factors, after removing the spatial gradient. Bacterial families are represented by blue arrows. The abbreviations are as follows: Phyl: *Phyllobacteraceae*, Beij: *Beijerinkiaceae*, Oxal: *Oxalobacteraceae*, Brad: *Bradyrhizobiaceae*, Xant: *Xanthomonas*, Rhodos: *Rhodospirillaceae*, Burk: *Burkholderiaceae*, Rhiz: *Rhizobiaceae*, Legi: *Legionellaceae*, Alca: *Alcaligenaceae*, Rhodoc: *Rhodocyclaceae*, Acti: Uncultured *Actinobacteria*, Gemm: *Gemmatinoidetes*, Gall: *Gallionellaceae*, Acid: *Acidobacteria*, Sphi: *Sphingomonadaceae*, Other D: Other δ-*Proteobacteria*, Geob: *Geobacteraceae*, Bact: *Bacteroidetes*, Hydr: *Hydrogenophilaceae*, Clos: *Clostridiaceae*, Pept: *Peptococcaceae*, Myco: *Mycobacteraceae*, Desu: *Desulfovibrionaceae*, Alic: *Alicyclobacillaceae*, Phyl: *Phyllobacteraceae*. Only bacterial families with a cumulative fit ≥5% on the first two axes are shown. Environmental variables are represented by arrows, except for engineered controls, which are expressed in nominal variables and are represented by triangles. The projected location of each bacterial population point along each arrow indicates its correlation to that environmental variable. The canonical axis explains 70.8% of the total variability in the bacterial community data (P=0.060 ).

**B.** Canonical correspondence analysis ordination diagram (biplot) summarizing the effects of environmental factors, after removing the spatial gradient. Samples are represented by open circles, the proximity of which indicates occurrence in similar environmental conditions. Engineered controls are represented by triangles. Other environmental variables are represented by arrows, which point toward increasing values of that variable. Their length is directly proportional to their importance in influencing the bacterial populations of that sample. The canonical axis explains 38.2% of the variance in the samples (p=0.0020).

## ACKNOWLEDGEMENT

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